

Structure investigations of 13.7 kD Basic Barley Protein
by two-dimensional ^1H NMR spectroscopy.

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With the aim to study the solution structure of a 13.7 kD Basic protein from Barley (8), BBP, two-dimensional ^1H NMR spectra (COSY, DQF COSY, Relayed COSY, TOCSY, Double Quantum spectroscopy and NOESY) have been recorded at respectively two pH values (4.2 and 7.0) and at two temperatures (295 K and 310 K). The analysis so far has resulted in sequence specific identification of 112 (out of 124 possible) residues. The use of conventional sequence specific assignment procedures in the study of relatively large proteins is hampered by the broad resonance lines. However many problems have been solved by iteratively using structure information from regions of the protein which behave "well" with respect to produce well resolved NMR spectra together with NOE effects from regions which were less well resolved to sort out ambiguities in the NMR analysis.

The most significant secondary structure element in this protein is a four stranded anti-parallel beta sheet which involves 38 residues (residue 1 - 9, 65 - 81 and 113 - 124). Furthermore a small parallel beta sheet has been found (residue 49 - 51 and 84 - 86). Only small fractions of alpha helix like secondary structure elements have been found (residue 26 - 30, 31 - 37, 99 - 103 and 107 - 113). So far (90 % assigned) only half the protein consists of regular secondary structure elements. Protons in several regions of the protein gave relatively broad resonance lines especially the lines of amide protons were broad and therefore difficult or impossible to assign. This has been interpreted as regions on the surface of the protein, which seems to agree well with the preliminary structures.

The analysis of the NOESY spectra have resulted in 405 distance restraints and analysis of COSY have given 107 phi angle restraints. This information has been used to calculate the three dimensional structure initially using Metric Matrix Distance Geometry (1,2,3) and further refinements by using Restrained Energy Minimization and

Restrained Molecular Dynamics (4,5,6).

Calculations were firstly performed only for the 38 residues in the four stranded anti-parallel beta sheet. This was inspired by the fact that for these residues the number of assigned NOE effects was relatively large (155 NOE's), and it was possible to determine almost all phi angles. The structures, especially those from the Restrained Dynamics calculations, agreed very well with the input restraints, in fact several of the structures were fully in accordance with the input restraints. The average RMS differences between NOE restraints and interproton distances in 10 structures was $0.09 \pm 0.01 \text{ \AA}$. All the dynamics and minimizations were performed using XPLOR (7).

Very recent calculations for the entire structure of the 124 residue large protein (five structures by Restrained Molecular Dynamics) show RMS differences between the interproton distances and NOE restraints at $0.11 \pm 0.01 \text{ \AA}$, and the RMS difference between the backbone atoms and their average structure is $3.22 \pm 0.45 \text{ \AA}$ which is quite satisfactory.

The preliminary structure achieved for BBP possess several interesting structural features on the surface with two closely spaced histidines in the vicinity of three tyrosines and two thryptophans, which is a potential binding/catalytic site.

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